**Modulation of P2X4 receptor activity affects the expression and release of pro- and anti-inflammatory cytokines by mononuclear phagocytes**

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Pro-inflammatory cytokines that regulate innate immunity can be induced by pathogen-associated molecular patterns such as lipopolysaccharide (LPS) and play essential roles in host defense against infection. In response to a number of stimuli, including major surgery and accidental trauma, damaged cells release ATP, inducing P2X7 receptor (P2X7R) activation in innate immune cells. This leads to the release of a large number of cytokines, including the pro-inflammatory cytokine interleukin (IL)-1β, which can cause life-threatening, systemic inflammation. Despite the well-recognized role of the P2X7R in the assembly of the NLRP3 inflammasome and IL-1β release, it remains unclear whether other purine receptors can contribute to or modulate this inflammatory process. In this study, we examine the role of P2X4R in cytokine secretion and biosynthesis. Human monocytic THP1-cells, THP1-cell-derived macrophages, primary human peripheral blood mononuclear cells (PBMCs), human macrophages derived from PBMCs, and peritoneal human macrophages were primed with LPS. Cells were then left untreated or treated with a panel of P2X4R or P2X7R antagonists and the expression and release of pro-inflammatory (IL-1β, IL-6) and anti-inflammatory (IL-10) cytokines, as well as expression of P2X4R and P2X7R was analyzed by real-time RT-PCR, Western blot and ELISA. LPS-primed cells were also stimulated with ATP to induce secretion of NLRP3 inflammasome-dependent (IL-1β, IL-18) cytokines, which were measured by ELISA. Our data show that both the P2X4R and P2X7R seem to be involved in the ATP-induced IL-1β release by multiple types of mononuclear phagocytes, a typical hallmark of traumatic cell damage. Specifically, our data show that modulation of P2X4R activity can affect the LPS-induced expression and release of both, the NLRP3-dependent (IL-1β, IL-18) and -independent cytokines (IL-6, TNFα, IL-10). We identified different sensitivities and responses to P2X4R inhibition in monocytes relative to macrophages that may be explained by a 160-fold higher expression level of P2X4R in monocytes. These findings highlight an unexpected role of P2X4R in the expression and release of cytokines by mononuclear phagocytes. We propose that this paves the way towards novel therapeutic strategies protecting surgical and traumatized patients against infection, while efficiently preventing ATP-mediated hyperinflammation.

**Purinergic and neuroinflammatory genes involved in lithium treatment response in bipolar disorder**

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**Background.** For 70 years lithium is a first-line option for maintenance treatment in bipolar disorder (BP), but only 30% of patients have a long-term favorable response. Purinergic signaling was suggested as mediator of the biological effects of lithium (Gubert et al., 2021, Illes et al., 2022*)* by its neuroinflammatory/neuroprotective effect (Di Virgilio, et al., 2018). Lithium has anti-inflammatory properties by the inhibitory effects exerted on the GSK-3β enzyme and on the nuclear factor NF-κB pathway with consequent reduction of the pro-inflammatory cytokines TNF-α and IL-6 levels (Le Clerc et al., 2021). The International Consortium on Lithium Genetics that includes Romania investigated the genetic mechanisms of lithium long-term treatment response in BP in several studies. **Methods.** 2586 BP patients (164 Romanian BP-I) with minimum 2-year lithium treatment were genome-wide genotyped and their response was clinically assessed with the Alda scale. Functional pathway analysis was performed. **Results**. We showed that the purinergic receptor P2Y1 (P2RY1), a protein-coding gene with purinergic signaling role through receptors for extracellular ATP, the ATP2B2 gene with plasma membrane calcium-transporting function, and TNFRSF21 (Tumor necrosis factor receptor 21), as well as HLA antigen genes and genes encoding pro-inflammatory cytokines are involved in the treatment response to lithium in BP. They interact with schizophrenia genes. TNF, interleukin4(IL-4), and interferon-gamma (IFNγ) might represent important molecular nodes in the interaction between response to lithium and schizophrenia-SNP-loading. (Amare et al, 2018; Stone et al, 2021, Le Clerc et al., 2021). Schizophrenia-SNP-loading is inversely correlated with the response to mood stabilizers and antipsychotics. Moreover, we showed that a pathway controling the immune system (*REGULATION_OF_IMMUNE_SYSTEM_PROCESS)* (7 genes) is also involved in psychosis in BP (Grigoroiu-Serbanescu et al, 2024) and psychosis is a common phenotype for BP and schizophrenia. **Conclusion**. The purinergic genes and receptors are currently pharmacological targets for the treatment of mood disorders.

**The ATP receptor P2X7 mediates cell death in effector-prone human unconventional and tissue-resident T lymphocytes**

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Extracellular ATP is released by cells under inflammatory conditions and activates the P2X7 receptor. Dependent on the ATP concentration, P2X7 activation in T cells either promotes T cell activation or mediates susceptibility to ATP-induced cell death. In mice, P2X7 is highly expressed in tissue-resident memory T cells (TRM) cells, and blockade of P2X7 prevents ATP- and $NAD^+$-induced death of these cells. A detailed analysis of P2X7 expression and function in the human T cell compartment has, however, not been performed. In this study, we used a P2X7-specific nanobody with blocking capacity to assess protein expression and function of the P2X7 receptor on different human T cell subsets. Next to peripheral blood, we analyzed pediatric liver samples to analyze the expression of P2X7 in human TRM cells. We show that, within the human T cell compartment, unconventional T cells and liver TRM cells have the highest expression of P2X7. Both populations are ready-to-act T cells with high effector potential, and their homeostasis must be tightly regulated to prevent immune pathology. Using Tγδ cells as example for an unconventional T lymphocyte population, we demonstrate that these cells are more sensitive to P2X7 receptor activation than conventional T cells, affecting fundamental cellular mechanisms, like calcium signaling and ATP-induced cell death. Similarly, high P2X7-expressing liver TRM cells are susceptible to cell death after ATP treatment. Furthermore, we found that IL-15, a growth factor enhancing differentiation of TRM-like cells *in vitro*, increases P2X7 expression in different T cell subsets. The increased susceptibility of effector-poised T cells to P2X7-mediated cell death may provide a mechanism to control their homeostasis under inflammatory conditions.

**Exosomes derived from breast cancer MDA-MB-231 cell line express the purinergic receptor P2X4 and induce immunotolerant monocytes-derived dendritic cells by decreasing the surface expression of HLA-DR and glycogen content**

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Tumors are well known to promote the induction of immunosuppressive microenvironment by recruiting the surrounding cells to serve their own survival and growth. This could be assigned to the release of soluble factors by tumor and large numbers of heterogenic extracellular vesicles (EVs) of different origins represented in microvesicles and exosomes. Tumor-derived EVs gain the attention of several research groups in terms of their immunomodulatory effect as it encompass immunosuppressive proteins and microRNAs targeting suppressive pathways in recipient cells. This finding prompted us to investigate the immunomodulatory effect of exosomes derived from metastatic breast cancer MDA-MB-231 cell line expressing the purinergic receptor P2X4 on the differentiation of primary peripheral blood $CD14^+$ monocytes into immature dendritic cells (iDCs). To address this topic, we have isolated PBMCs from blood of healthy donors and then we selected $CD14^+$ monocytes. $CD14^+$ cells were treated with exosomes derived from the *wild-type* MDA-MB-231 cells that express the purinergic receptor P2X4 (MDA-EXO +/+P2X4). The cells were incubated for 7 days at 37°C and 5% $CO_2$ in the presence of IL-4 and GM-CSF. FACS analysis revealed that $CD14^+$ cells treated with MDA-EXO +/+P2X4 showed a significant reduction in the expression of the cell surface receptor HLA-DR compared to the positive control (cells only treated with IL-4 and GM-CSF). In addition, no significant differences regarding the surface expression of CD86, CD33, and CD14 as well as the expression of the released cytokines were observed. Interestingly we found that $CD14^+$cells treated with MDA-EXO +/+P2X4 displayed a significant reduction in the glycogen content compared to positive control using electron microscopy. We are planning to test the same experiments by treating $CD14^+$ cells with MDA-EXO deprived of the P2X4 receptor (MDA-EXO -/- P2X4) in order to investigate whether P2X4 plays a central role in the induction of immunosuppressive environment. Our results may provide new insight toward the immunosuppressive as well as the immunomodulatory role of tumor-derived exosomes.

**Purinergic signaling in the auditory brainstem during postnatal development of mice exposed to antenatal dexamethasone treatment**

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Nearly 1 million babies die each year from complications caused by premature birth. To prevent that, pregnant women at risk of preterm delivery between the 24th and 34th week of pregnancy are given a course of antenatal steroids (ANS). Despite recommendations, treatment is often repeated, frequently resulting in lifelong psychological and neurosensory impairments in the surviving infants.

In the auditory system, repeated ANS treatment (rANS) is associated with hearing impairment in children, as well as elevated hearing thresholds and changes in auditory brainstem response in rats. During auditory brainstem development, ATP signaling via purinergic P2X2/3 receptors increases the fidelity of the first central synapse, while the role of P2Y1 signaling remained unclear. We demonstrated upregulation of CD39 and CD73 in the fetal rat brain after rANS. Therefore, we hypothesized that rANS might impair auditory brainstem development by reducing ATP-, ADP-, and adenosine-mediated signaling.

We examined the effects of rANS on the auditory brainstem of C57Bl/6 mice at postnatal days P8, 14 and 20, which correspond to pre- and post- hearing onset, as well as juvenile stage, respectively. Pregnant dams received 0.4 mg/kg dexamethasone (DEX) s.c. on gestation days (GD) 15-17 to mimic repeated clinical treatment for three consecutive weeks. The control group (Sh) received saline.

DEX treatment induced transient decrease in P2Y1 mRNA expression in P8, while a developmental decrease was observed for P2X2. While the expression of CD39 was stable, the expression of NTPDase2 changed during development in parallel to P2Y1 receptor in a monophasic manner, reaching its maximum at P14. Both CD73 and the A1 receptor showed a developmental increase in the expression from P8-P20, unaffected by a treatment.

Parallel changes in the expression of the components of the ADP signaling system, particularly pronounced around the onset of hearing, indicate the developmental role of ADP signaling in the auditory brainstem, while transient decrease in the expression of the P2Y1 receptor induced by rANS may have functional consequences that remain to be elucidated. Parallel increase in the expression of CD73 and A1 receptor from P8-P20 underscore the importance of adenosinergic signaling in brainstem maturation during early postnatal development.

**Circulating miR-22 as a biomarker of hippocampal P2X7 overexpression and drug-refractoriness in patients with mesial temporal lobe epilepsy (MTLE)**

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Mounting evidence suggest that ATP-gated ionotropic P2X7 receptors (P2X7R) actively participate in epilepsy and other neurological disorders. Neocortical nerve terminals of patients with Mesial Temporal Lobe Epilepsy with Hippocampal Sclerosis (MTLE-HS) exhibit higher density of P2X7R than control samples. Overexpression of P2X7R bolsters ATP signals during seizures resulting in glial cells activation, cytokines production and GABAergic rundown with unrestrained glutamatergic excitation. In a mouse model of status epilepticus, the P2X7 overexpression is associated with down-modulation of the non-coding micro RNA, miR-22. MiR levels are stable in biological fluids and normally reflect remote tissue production making them ideal disease biomarkers. Here, we compared P2X7R and miR-22 expression in epileptic brains and in the serum of patients with MTLE-HS, respectively. The expression of P2X7R in the hippocampus and anterior temporal lobe of 23 patients with MTLE-HS and 10 cadaveric controls was performed by quantitative RT-PCR. Confocal microscopy and Western blot analysis was performed to assess P2X7R protein amounts. MiR-22 expression was evaluated in cell-free sera of 40 MTLE-HS patients and 48 healthy controls. The results show that nerve terminals of the hippocampus and neocortical temporal lobe of MTLE-HS patients overexpress ($p<0.05$) an 85kDa P2X7R protein whereas the normally occurring 67kDa receptor protein dominates in the brain of cadaveric controls. Contrariwise, miR-22 serum levels are diminished ($p<0.001$) in MTLE-HS patients compared to age-matched control blood donors, a situation that is more evident in patients requiring multiple (>3) anti-epileptic drug (AED) regimens. Data suggest that there is an inverse relationship between miR-22 serum levels and P2X7R expression in the hippocampus and neocortex of MTLE-HS patients, which implies that measuring serum miR-22 may be a clinical surrogate of P2X7R brain expression in MTLE-HS. Moreover, the high area under the ROC curve (0.777; 95% CI 0.629 to 0.925; $p=0.001$) suggests that low miR-22 serum levels may be a sensitive predictor of poor response to AEDs among MTLE-HS patients. Results also anticipate that targeting the miR-22 / P2X7R axis may be a good strategy to develop newer AEDs. This work was funded by FCT (POCTI PTDC/SAU-PUB/28311/2017 - EPIRaft grant; MedInUP -UIDB/04308/2020 and UIDP/04308/2020; UMIB - UIDB/00215/2020 and UIDP/00215/2020; ITR - Laboratory for Integrative and Translational Research in Population Health - LA/P/0064/2020).

**P2X7R and P2X4R expression in the retina of mouse models of Central Areolar Choroidal Dystrophy (CACD)**

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The purinergic receptors P2X7 (P2X7R) and P2X4 (P2X4R) are involved in the glial activation and neuroinflammation that is present in all retinal neurodegenerative diseases, as well as in the calcium overload leading to apoptosis and cell death. In previous works, we have found increased expression of both P2X7R and P2X4R in THE RETINA OF different models of retinal dystrophies, either due to mutations affecting the phototransduction process or to the structure of the photoreceptors. Using mouse models ofcentral areolar choroidal dystrophy (CACD), with mutations affecting the PRPH2 gene, that codes for the photoreceptor´s structural protein peripherin, we have analyzed by flow cytometry the P2X7R and P2X4R expression in THE RETINA OF homozygous or heterozygous Prph2 Knock-in and Knock-out models at the same age, and in the $Prph2^{KI/WT}$ along the progression of the disease. We have found that retinas from homozygous Prph2 Knock-in and Knock-out mice show a higher expression of P2X7R and P2X4R than the heterozygous phenotypes and wild type mice. In the retinal cells of $Prph2^{KI/WT}$ mice, there is an age- dependent increase in the population of cells expressing P2X7R and P2X4R from 3 months of age onward with a transient peak of expression at one month of age. Several CELL populations with different levels of expression of CD11b, P2X7Rand P2X4R were observed. Our present work is focused on the contribution ofP2X7R and P2X4R to each phase of the retinal degeneration to further analyze their possible use as therapeutic

**Mitochondrial localization of adenosine receptors modulates energy metabolism**

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G-Protein Coupled Receptors (GPCRs) are classically considered cell-surface receptors that transmit extracellular signals into cells through different effector systems and second messenger generation. Adenosine receptors belong to Class A of GPCRs and are widespread throughout the body where they perform many biological functions depending on the specific tissue. There are four subtypes of adenosine receptors: $A_1$ and $A_3$ are coupled to Gi-proteins and $A_{2A}$ and $A_{2B}$ are coupled to Gs-proteins modulating cAMP levels. The aim of this study was to examine whether adenosine receptors are localized in the mitochondria from several tissues. For this purpose, a highly purified mitochondrial fraction from mouse and human brain, mouse liver and HeLa cells was obtained by Percoll-gradient ultracentrifugation. Data from Western blots revealed the mitochondrial localization of adenosine $A_1$, $A_{2A}$ and $A_{2B}$ receptors. Moreover, electron microscopy analysis, considered the "gold standard" method for intracellular localization of receptors, and radioligand binding assay further corroborated the mitochondrial localization of adenosine receptors in mouse brain and liver, respectively. Regarding adenosine signalling in the mitochondria, these receptors appeared to be coupled to their canonical G-proteins in this organelle as reported in plasma membrane. A low activity of CD73, an adenosine-generating enzyme, was also detected. Mechanistically, Seahorse analysis revealed that exposure to adenosine receptor agonists modulated energy metabolism in isolated mitochondria from HeLa cells. Regarding mitochondrial morphology, Mitotracker Green staining revealed a complex response upon pharmacological stimulation of adenosine receptors. Our results suggest that mitochondrial localization of adenosine receptors may modulate the activity of this organelle and affect its morphology. Therefore, the role of these receptors on mitochondrial dysfunction, a distinctive hallmark of several pathologies, should be considered.

**Study of the ionotropic purinergic receptors during human cortical development using a brain organoid model**

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The development of the human cerebral cortex is a process that involves a sequence of closely coordinated genetic, physical, biochemical, and environment-associated events. However, the study of the molecular mechanisms that control human corticogenesis has, to date, been limited, largely due to the scarcity of suitable models. In response to these limitations, the brain organoid model has emerged as an excellent tool to study the molecular mechanisms involved in the proliferation, differentiation, and migration of human neural stem cells and their progeny, since they recapitulate a large part of the phases of human embryonic cortical development. Regarding these modulatory mechanisms that govern the stages of cortical formation, purinergic receptors are postulated as promising candidates since they play a key role in the development of the nervous system. However, the knowledge about the role of this signaling system in the embryonic development of the human cerebral cortex is still scarce. Consequently, our research focuses on characterizing the expression and function of purinergic receptors during the development of the human cerebral cortex based on the brain organoid model. The data obtained so far demonstrate significant expression of ionotropic purinergic receptors within the progenitor domain as well as in immature neurons in organoid cortical tissue. We also found that ionotropic receptors were functional and that their pharmacological inhibition had a determinant effect on the development of organoid neural ventricles. These results will help us gain a more detailed understanding of the role of ionotropic purinergic receptors in the regulation of human cortical architecture, identifying potential candidates for the design of therapeutic strategies.

**The role of extracellular ATP signalling in chronic stress and cancer**

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Chronic stress can contribute to or exacerbate many diseases, including cancer. In recent years, we have demonstrated the key role of macrophages and their tissue-specific interactions with mesenchymal stem cells (MSC) in regulating stress erythropoiesis (SE), focusing particularly on the involvement of nitric oxide (NO) and purinergic signalling in these interactions.

As an experimental model of chronic psychological stress, we used an animal model of restraint stress (male BALB/c mice) as well as mono- and co-cultures of mouse macrophages (RAW264.7) and MSC isolated from mouse bone marrow and spleen. *In vivo*, macrophage depletion was performed by intraperitoneal administration of clodronate liposomes before and during stress treatment to remove resident macrophages. *In vitro*, cell mono- and co-cultures were treated with erythropoietin and corticosterone (SE mediators), a nitric oxide synthase (NOS) inhibitor and/or a CD73 inhibitor (APCP).

The results showed increased extracellular ATP (eATP) levels after chronic stress, upregulated P2X7R expression, and increased activity and expression of CD39 in the bone marrow and spleen, while depletion of macrophages abolished these stress effects. Expression of genes for ADORA2A and ADORA2B receptors was also increased by chronic stress treatment, but macrophage depletion only reversed the upregulation of ADORA2A receptor expression. Chronic stress did not affect the activity and expression of CD73 in the bone marrow and spleen, while macrophage depletion increased only enzyme activity. *In vitro*, SE mediator treatment significantly increased eATP production in co-cultures of bone marrow-derived MSC and RAW264.7 cells, while NOS blockade abrogated this stress-induced effect and increased CD73 gene expression. When MSC from the spleen were co-cultured with RAW264.7 cells, the SE mediators together with NOS blockade led to increased eATP release. Inhibition of CD73 enzyme activity in co-cultures treated with SE mediators further reduced NOx production and significantly affected NOS enzyme expression. Treatment with SE mediators led to a decrease in adenosine concentration in the co-culture of bone marrow/spleen-derived MSCs and RAW264.7 cells, and NOS blockade had no effect on this decrease. The results obtained provide a new insight into the complex mechanisms regulating erythropoiesis under stress conditions and may contribute to the development of new and more effective treatments for anemia.

Since cancer-related anemia is common in cancer patients, we have extended the research topic to the effects of chronic stress on primary tumor progression, metastasis and the tumor-altered bone marrow and spleen microenvironment. For this new research, we used an animal model of triple negative breast cancer (BALB/c – 4T1 breast cancer model) and *in vitro* co-cultures (RAW 264.7 and 4T1 breast cancer cell line). Preliminary *in vitro* results indicate an interplay of NO and purinergic signalling in the interaction between macrophages and tumor cells.

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**Pancreatic cancer resistance to immune checkpoint blockade is rescued by depletion of extracellular ATP in an orthotopic murine model**

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Immune checkpoint blockade (ICB) therapies have redefined the way neoplastic diseases are treated. However, pancreatic ductal adenocarcinoma (PDAC) is one of the few tumor entities in which immunotherapies have shown no to little efficacy with a 10-year survival rate that remains abysmal at approximately 1%. Recent studies have shown a pivotal role played by the intestinal microbiota in dictating response to immunotherapies. This is extremely relevant for PDAC as the intestinal and pancreatic lymphatic network is extensively shared, suggesting that manipulating the intestinal environment may improve the efficacy of immunotherapies. In our laboratory, we have shown that depletion of the intestinal extracellular ATP (eATP) through the oral administration of the apyrase enzyme, generates a secretory IgA (sIgA) response that could restore intestinal homeostasis during pathological conditions. Using a clinically relevant orthotopic murine model of PDAC, we found that we could improve ICB efficacy by concomitant intestinal eATP depletion. Therapeutic benefit was associated with an increase in intratumoral germinal centre-like $IgA^+$ B cells and □□ T cells expressing CXCR5. Importantly, such effect was IgA-dependent as therapeutic effect was lost in IgA knockout mice. Further characterization of the $CXCR5^+$ □□ T cells revealed that these cells are microbiota-dependent, have the ability to induce IgA responses *in vitro*, and are extremely sensitive to ATP-induced cell death compared to conventional □□ T cells. Altogether, our data suggest that intestinal eATP depletion boosts the efficacy of ICB against PDAC, at least in part, by increasing the fitness and activity of a □□ T cell population endowed with the ability to elicit antitumor IgA responses.

**Purinergic system modulates hippocampal synaptic activity following repetitive transcranial magnetic stimulation**

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Intermittent theta burst stimulation (iTBS), a specific excitatory protocol of repetitive transcranial magnetic stimulation, provides painless and non-invasive brain stimulation and has shown promising therapeutic effects in a variety of neurological and neuropsychiatric disorders in both animal studies and clinical trials. Nevertheless, the underlying mechanisms by which iTBS exerts its efficacy and produces lasting effects are still largely unknown, limiting the understanding of the therapeutic benefits and potential optimization of the protocol. To investigate the effects of iTBS on the synaptic compartment of hippocampus of healthy animals, two-month-old Wistar rats were used and treated with either iTBS or sham stimulation twice daily for seven days. After the last day of stimulation, animals from both groups were sacrificed and synaptic compartments *i.e.* synaptosomes were isolated and used for further analysis. Immunoblot analysis of hippocampal synaptosomes revealed high expression of P2X7 receptors ($p > 0.9$), but no changes were observed after iTBS, while increased expression of CD73 ($p < 0.05$), A1R ($p < 0.05$), A2AR ($p < 0.05$) and ADA1 ($p < 0.05$) in the iTBS group compared to the Sham group were detected. The changes in protein expression were also followed by increased AMPase activity in the iTBS group (67.09 ± 9.17 nmol Pi/mg/min, $p < 0.01$) compared to the sham group (50.92 ± 7.95 nmol Pi/mg/min, $p < 0.01$). Interestingly, this increase was also followed by an increase in ADA activity in the iTBS group (31.95 ± 6.1 nmol NH4+/mg/h, $p < 0.05$) compared to the sham group (23.16 ± 6.43 nmol NH4+/mg/h). Together with other data, it appears that prolonged iTBS favors presynaptic A1R activation as a gatekeeper of excessive activation, since iTBS is an excitatory protocol, whereas the A2AR receptor may contribute to the LTP-like changes observed after iTBS.

**Purinergic Signalling in intracellular pathogens infection: The role of P2X7 receptors**

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P2X7 receptor emerged as a critical purinergic receptor involved with recognising and triggering first immune responses during intracellular parasite infections, particularly during intracellular bacteria and protozoans infections. The mechanisms involved go from phagolysosomal fusion and acidification, intracellular calcium mobilisation, ROS production, leukotriene B4 and cysteinyl leukotrienes production and secretion, NLRP3 inflammasome assembly and IL-1β secretion, apoptosis and pyroptosis. Furthermore, it was recently shown that P2X7 receptors participate in recognising and shaping the immune response against virus infections. It was shown that viral infection can induce ATP release to the extracellular environment and P2X7 receptors positive modulation. Recently, we investigated the role of ATP-P2X7 receptor signalling in Zika-related brain abnormalities and Covid-19. Our findings suggest that ATP-P2X7 receptor signalling contributes to the antiviral response in the brain of ZIKV-infected mice while increasing neuronal loss, neuroinflammation, and related brain abnormalities. It is likely that P2X7 receptors also contribute to poor COVID-19 outcomes since serum from patients with COVID-19 has increased levels of eATP compared to control individuals, and it can contribute to the cytokine storm associated with severe COVID-19.

Considering that P2X7 receptors are the primary purinergic receptors involved in neuroinflammation, neurodegeneration, and immunity, the new findings of how these purinergic receptors contribute to sensing induce immune response is critical to a better understanding of how to deal with intracellular pathogens-induced diseases.

**Purinergic control of synaptic activity under hyperexcitable pathological conditions**

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ATP is released by neurons and astrocytes as a function of neuronal activity. ATP can then be metabolized into adenosine to fine tune synaptic signalling. Under physiological conditions the adenosine is then transported back to the cytoplasm through equilibrative adenosine transporters, and then converted into AMP by adenosine kinase (ADK) that keeps the intracellular adenosine concentrations below the extracellular ones. However, as I shall discuss, this scenario may change under pathological conditions.

When ADK levels are pathologically increased, the resulting extracellular adenosine deficiency and consequent decreased tonic activation of inhibitory A1R, translates into increased susceptibility to seizures[1]. However, ADK deficiency also leads to seizure susceptibility, which likely results from chronic overexposure to extracellular adenosine, decreased $A_1R$ expression[3] and adenosine $A_{2A}R$-dependent exacerbated facilitatory effects of BDNF on hippocampal synapses[4]. In contrast, in neurodevelopmental disorders, as RETT syndrome, where BDNF and adenosinergic signaling are hypofunctional, co-activation of $A_{2A}R$ and TrkB receptors may rescue synaptic dysfunctions[5].

A role for $A_3R$ is much less known and being even controversial in epilepsy[6]. Being intrigued by the finding that a moderately $A_1R$-selective agonist, MRS5474, suppresses clonic seizures in a mouse model without appreciable cardiac action[7], we hypothesized that this drug could act through other than A1R and/or through a brain specific mechanism. We thus assessed the effect MRS5474 at the hippocampus[7]. MRS5474 inhibited excitatory transmission under hyperexcitable conditions but not in control ones, precluding an $A_1R$ mediated action. MRS5474 inhibited GABA uptake in hippocampal slices, an effect likely mediated by adenosine $A_3R$ since it was prevented by an $A_3R$ antagonist and mimicked by an $A_3R$ agonist. Importantly, the expression of $A_3Rs$ is increased in epileptic tissue from the human hippocampus, and MRS5474 enhanced GABAergic currents in human tissue taken from the human epileptic hippocampal tissue but not from control tissue.

Summarizing, $A_1Rs$ play a major role in control of excitability under physiologic and some pathologic conditions, but $A_3Rs$ may play an additional anticonvulsant action under epileptic conditions. $A_{2A}Rs$ increase seizure susceptibility under chronically enhanced adenosinergic tonus.

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**P2X4 receptors in ALS and its potential use as biomarker for the diagnostic**

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by the selective loss of motor neurons (MNs). P2X4 receptor which is an ATP-gated cation channel has been recently involved in ALS pathogenesis using the ALS SOD1G93A (SOD1) mice. Using double transgenic SOD1 mice expressing either a general surface increase (P2X4KI) or deletion of P2X4 (P2X4KO), we surprisingly revealed that both genotypes resulted in improved ALS symptoms and mouse lifespan. These paradoxal results may point out a complex cell-specific function of P2X4. To address the neuroglial role of P2X4 in ALS, we developed novel triple transgenic SOD1 mice inducing either an increase in surface P2X4 or a deletion of P2X4 gene selectively in microglia/macrophages or neurons. We show the neuronal increase of P2X4 accelerates ALS progression, spinal MN degeneration and mouse death while increase of P2X4 at the surface of microglia/macrophages has a positive impact on the progression of the disease. We are currently studying morphological and functional changes in microglia from these lines which may support neuronal viability through ALS progression. Further, we have previously showed that misfolded proteins such as mutant SOD1 increase surface P2X4 trafficking in the spinal cord of ALS SOD1G93A mice. Interestingly, surface increase of P2X4 was also observed in peripheral macrophages of SOD1 mice even before the symptom's onset and during ALS progression. We thus developed a method to measure by flow cytometry the surface-to-total ratio of P2X4 in monocytes from human peripheral blood samples. Our data reveal that surface density of P2X4 is significantly higher in monocytes of ALS patients, positioning P2X4 as a biomarker to help ALS diagnosis. This work may provide valuable insights into the cellular role of P2X4 to fight this fatal disease, but also defines P2X4 as a promising ALS biomarker.

**Description of a patient with autism, neurodevelopmental delay, absent serum IgA and enrichment of Lactobacilli in gut microbiota, associated with rare biallelic variants of *P2X7R*.**

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The patient was born at term to non consanguineous parents, with birth weight 2400 gr. (do tue intrauterine growth retardation). Speech and motor development were delayed; she sat at 15 months and walked independently at 28 months. Impaired motor coordination with frequent falls but normal muscular tone and reflexes, no developmental regression or seizures were present. Her behaviour was caracterized by hyperactivity and autistic spectrum disorder. She suffers from recurrent respiratory infections, which prompted immunological work-up revealing absent serum IgA. Neuroimaging showed cerebellar vermis hypoplasia and thin posterior corpus callosum. She also presented a thyroglossal duct cyst and borderline glaucoma. Whole Exome Sequencing detected biallelic rare variants of P2X7R: p.R53W and p.N195I, each inherited from one healthy parent and encoding the ATP-gated ionotropic P2X7 receptor, expressed in immune cells, glia and neurons where it may play a detrimental or beneficial role, according to the activation of ATP dependent biochemical pathways, In P2rx7-/- mice,T follicular helper cells expansion in the Peyer's patches of the small intestine, germinal centre reaction and IgA secretion are dysregulated. The secretory immunoglobulin A in mammalian gut protects the organism from infections and contributes to host physiology by shaping microbiota composition. The microbiomic analysis of the patient showed a severely altered profile (49% dysbiosis) with predominance of Lactobacilli populations as described in the model mice. Patient derived fibroblast will be analyzed to explore impact of the variant of P2X7 receptor function. Modulation of P2X7R activity may pave essential directions toward therapeutic interventions in this patient.

**Effects of repetitive magnetic stimulation on purinergic signaling system in activated spinal cord mixed glial cells**

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Spinal cord injury (SCI) initiates a cascade of events leading to chronic inflammation and limited functional recovery. SCI is marked by the infiltration of inflammatory cells, the release of cytokines facilitating injury and glial scar formation. Reactive astrocytes and microglia, play critical role in neural plasticity and regeneration after SCI. Therapeutic strategies targeting the modulation of the glial scar and inflammatory response are essential for enhancing the recovery potential. Repetitive magnetic stimulation (rTMS) has shown significant therapeutic potential for SCI by promoting neural plasticity and functional recovery, and by inhibiting neurotoxic polarization of astrocytes, reducing neuroinflammation, by enhancing cortical excitability and facilitating neural connections through long-term potentiation. These findings suggest that rTMS could be a valuable intervention for enhancing recovery after SCI, but cellular and molecular mechanisms of rTMS affecting glia directly are yet to be investigated.

The objective of this study was to analyze the effects of rTMS on purinergic signaling system in activated mixed glial cells (mice spinal cord microglia and astrocytes), specially the role of ectonucleotidases, P1 and P2 receptors changes and regulation *in vitro*. To model the SCI in mixed glial culture monolayer, we utilized the scratch wound assay with the LPS addition to enforce astrocyte and microglia activation, followed with quadruple rTMS protocol. The cells and supernatant were collected after 24 h, and analyzed broadly. The preliminary results indicate that rTMS supports wound closure in scratch assay, with the higher cell velocity in the first 8 hours, during and immediately after the rTMS protocol, compared to appropriate controls. rTMS strengthens the antioxidative protection, attenuates astrocyte and microglia reactivity and changes the purinergic components expression. Most interesting is the downregulation of the P2X7 receptor, which consequently could lead to lesser inflammasome activation and less IL-1β release. Additionally, our research shows that rTMS induces ecto-5'-nucleotidase/CD73 increase, directly affecting the adenosine production, and thus, having more of the adenosine in the extracellular milieu. Further analysis of other P2X and adenosine receptors would reveal the potential mechanism of anti-inflammatory effect underlying the rTMS protocol therapeutic potential.

**New approaches for accelerating ligand development for P2X receptors**

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Generating selective ligands for P2X receptors takes a huge effort from discovery, characterisation, and structure-activity relationships before we can use them in cell assays and gather preclinical data. Often we know very little about ligand binding sites unless a crystal structure or a cryoEM structure is solved with the ligand bound and we have very few of these available to us within the purinergic field. Together with expert collaborators my team has developed a new approach to studying ligand binding at P2X receptors to gather key contact information about the ligand-protein interaction. This could provide important insights for making new chemical analogues. Through the use of binding site mutants or different P2X proteins from multiple species, differences in ligand interactions can be measured rapidly using saturation transfer difference NMR spectroscopy on living cells. This has the added advantage that ligand binding information is measured while the protein is present in a native environment rather than in an artificial membrane. Furthermore, the P2X protein is unmodified and the cell line can be used to generate complementary pharmacological data. This experimental approach can then be combined with computational docking to visualise predicted ligand poses based on real experimental data. This could be hugely beneficial when designing or refining new allosteric modulators for any P2X receptor, speeding up the ligand development process. We will present ligand epitope data on two selective negative allosteric modulators of P2X7, AZ10606120 and JNJ47966567 plus an interesting modulator GW791343 which acts as a NAM at human P2X7 but has positive allosteric modulator activity at rat P2X7 potentially through the same binding site. We will also present data on BAY1797 interactions at human P2X4 complementing the recent cryoEM structure and some evidence for BAY1797 activity at P2X7 receptors.

**Role of ATP metabolism in neurovascular coupling and retinal function in the mammalian eye**

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ATP and adenosine have emerged as important signalling molecules in various organs and tissues, including eye. The involvement of purinergic signaling in vascular remodeling, retinal functioning and neurovascular coupling indicates the necessity of targeting this pathway in various ocular diseases. However, current knowledge on metabolic pathways governing the duration and magnitude of ocular purinergic signaling is incompletely understood. By employing three-dimensional multiplexed imaging, in situ enzyme histochemistry, scanning electron microscopy, single cell transcriptomics, and flow cytometric analysis, this study dissected cellular purine homeostasis as a complex and coordinated network. In particular, we identified several key components of ocular ATP turnover, which are selectively expressed in the photoreceptor layer (CD73), choroidal and retinal vasculature (CD39, Connexin-43), glia (NTPDase2, Connexin-43), microglial cells (CD39, P2Y12R) of both mouse and human eyes, and also in human vitreous fluid. The relevance of purinergic signaling in retinal function has been demonstrated in the experimental murine model of oxygen-induced retinopathy, as well as in mice with altered melanocyte development due to a single nucleotide mutation in the microphthalmia-associated transcription factor (MITF) gene. Our study thus identified the presence of an extensive and spatially arranged network of purinergic ectoenzymes and receptors in the mammalian eye, and further characterizes the critical role of the ATP-adenosine axis in maintaining the functional activity of retinal cells.

**Olfactory receptors, the unsuspected partners of adenosine receptors.**

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Microglia accumulate in Alzheimer's disease (AD) lesions. While adenosine $A_{2A}$ receptor expression is minimal in prefrontal cortex microglia, it is significantly elevated in AD patient samples. Adenosine $A_{2A}$ receptor-targeting drugs, approved in Japan and the US for Parkinson's disease treatment, might prevent neuronal death in neurodegenerative diseases. Part of our research focuses on microglia-mediated mechanisms of neuronal death prevention.

Olfaction has been crucial in evolution, explaining why olfactory receptors are the largest family in the human proteome, with around 400 OR genes expressed in the CNS sensory areas. Evidence suggests olfactory receptors have significant functions in non-sensory CNS areas and in the periphery, likely acting as chemoreceptors with yet-to-be-identified endogenous ligands.

Our interest in CNS "ectopically" expressed olfactory receptors arose from transcriptome data showing differential expression in activated microglia treated with adenosine receptor ligands. Additionally, we found that adenosine $A_{2A}$ receptor functionality is regulated by direct interaction with olfactory receptors. Our more recent results indicate that activated microglia markedly upregulate heteromeric complexes constituted by $A_{2A}$ and olfactory receptors.

# The role of P2X7 and P2Y2 receptors in mediating breast-to-brain metastatic transition and phenotypic plasticity

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Metastatic disease is the main cause of intracranial tumors. The brain is a first metastatic niche of recurrence after treatment of HER2+ or triple negative breast cancer subtypes. To successfully colonize the brain, in addition to crossing the blood–brain barrier, breast tumor cells must acquire GABAergic characteristics and establish a favorable interaction with cell types that occur exclusively in the brain. In breast-to-brain cells, the expression of GABAA receptors, GABA transporters, the enzyme GABA transaminase, and the GABAergic interneuron markers parvalbumin and reelin is increased compared to primary tumor cells. This phenotypic change, a phenomenon called the breast-to-brain transition, is a malignant adaptation necessary for metastasis in the brain. The aim of this study is to investigate the mechanisms by which purinergic signaling mediates neural differentiation and the phenotypic transition process in breast cancer cells, as well as to explore howchemotherapy and the tumor microenvironment induce such phenotypic transition. The results showed that the P2X7 receptor is expressed in both neural progenitor cells and tumor cells. We demonstrated that during neural differentiation, P2X7 receptor expression is suppressed; moreover, silencing of the P2X7 receptor by shRNAs played a crucial role in determining glial phenotypes in murine embryonic carcinoma cells in vitro. We demonstrated that the presence of P2X7 receptorisoform A in tumor cells that exhibit an undifferentiated phenotype is crucial for triggering neural-like differentiation, and silencing of this isoform results in resistance to retinoids. On the other hand, in mice, cancer cells expressing P2X7 receptor isoform B was associated with the shorter survival. This isoform B was also mainly related to drug resistance. During repeated rounds of chemotherapy, a chemoresistant population emerged, accompanied by a decrease in P2X7 receptor levels and an increase in P2Y2 receptor expression levels. Importantly, P2Y2 receptoractivation is involved in the differentiation of mouse embryonic stem cells into GABAergic neurons while reducing the percentage of other neuronal types. Based on these collective findings, we posit that P2X7 and P2Y2 receptors play complementary roles in driving phenotypic plasticity and cell differentiation, suggesting their potential exploitation in the development of novel therapies for breast cancer patients.

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**Restoring heme levels in cellular models of congenital sideroblastic anemia: the role of pyridoxal-5'-phosphate**

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SLC25A38 gene mutations are responsible for the second most common form of congenital sideroblastic anemias (CSA). Unfortunately, there is currently no cure for this severe form of anemia, and pharmacological studies have been hindered by the absence of suitable biological models. To address this limitation, we examined two human cell lines: K562 erythroleukemia cells with reduced expression of the SLC25A38 protein and a lymphoblastoid cell line derived from an infant carrying a nonsense mutation in the SLC25A38 gene. Both cell lines replicated the main features associated with this anemia, including reduced heme content and respiratory defect. On the contrary, other defects were specific to K562 mutant cells such as the increase in mitochondrial iron, ROS species levels, and sensitivity to oxidative stress. Interestingly, the study uncovered a new role for extracellular pyridoxal 5'-phosphate (PLP) and antagonists of P2 receptors in rescuing the altered parameters of mutant cells. We propose that P2 receptors might be a promising target for pharmacological interventions in CSA caused by mutations in SLC25A38 gene.

**Role of P2X7 receptor in pancreatic cancer – from cellular function to risk association studies**

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Pancreatic ductal adenocarcinoma (PDAC) has one of the highest mortality rates of all cancers with a 5-years survival rate of 12%. The cellular/molecular mechanisms that give rise to PDAC are still unresolved. The PDAC tumor microenvironment (TME) is acidic, fibrotic and extracellular ATP concentrations are presumably higher that in the physiological state. Our overall aim is to understand whether the P2X7 receptor (P2X7R) is involved and determines tumor development/progression. We have used a number of models including animal cancer models and in vitro cells analyses and found that P2X7R plays an important role in the crosstalk between pancreatic stellate cells (PSCs) and cancer cells, most probably promoting progression of PDAC. In our recent study, the aim was to investigate a possible association of human P2X7R SNP variants with the risk of developing PDAC. Control and PDAC subjects were genotyped for 11 non-synonymous SNPs in P2X7R. The analysis showed that two SNPs 474G>A and 853G>A (rs28360447, rs7958316) that lead to the Gly150Arg and Arg276His variants had a significant but opposite risk association with PDAC development, protecting against and predisposing to the disease, respectively. Hence, we performed functional studies on pancreatic cancer cells and PSCs expressing the two P2X7R variants and GFP and monitored intracellular $Ca^{2+}$ response, YO-PRO-1 dye uptake, cell migration and release of various cytokines. The Gly150Arg variant displayed LOF traits and correlates with a protective phenotype. The Arg276His variant had variable responses, depending on the functional assay, but some correlation with higher risk of developing pancreatic cancer could be predicted. In conclusion, we provide evidence for the association of P2X7R SNPs with pancreatic cancer and suggest that they could be considered as potential biomarkers.

**P2X4 receptor and prostate cancer**

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Prostate cancer (PCa) is the most frequently diagnosed cancer in men, causing considerable morbidity and mortality. The P2X4 receptor (P2X4R) is positively associated with tumorigenesis in many cancer types, but its involvement in PCa progression is less understood. We showed that P2X4R is the most highly expressed and functional P2 receptor in various PCa cell lines. Inhibiting P2X4R on PCa cells (PC3 and C4-2B4) using selective P2X4R antagonists 5-BDBD and PSB-12062 led to impaired growth and mobility of PCa cells but did not affect apoptosis. Daily intraperitoneal administration of 5-BDBD (10 mg/kg) exhibited anti-tumorigenic effects in BALB/c immunocompromised nude mice inoculated with human PC3 cells subcutaneously. Genetically knocking out P2X4R in PCa cells using the CRISPR/Cas9 system significantly reduced cell proliferation and invasiveness. In a xenograft model systemically inoculated with PC3 cells via the intracardiac route, BALB/c nude mice receiving P2X4R knockout (KO) cells showed slower tumour progression, protection from PCa bone metastases, and associated bone destruction. These results are further supported by our retrospective analysis of P2RX4 expression in clinical datasets (GDS1439, GDS1746, and GDS3289), suggesting that P2X4R is positively associated with PCa malignancy. Furthermore, RNA-Seq and bioinformatic analysis on P2X4R KO cells demonstrated links between P2X4R and PCa cell adhesion, Wnt signalling, and IL-2 production. These findings suggest that P2X4R could be a potential therapeutic target for the treatment of aggressive and metastatic PCa.

**Targeting ATP-mediated signaling in the tumor microenvironment as a supportive therapeutic approach**

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Extracellular ATP (eATP) is a dominating purine component in the tumor microenvironment (TME), and ATP-mediated signaling is known as one of key factors in cancer progression. Within the tumor cells niche, in addition to a non-regulated release of ATP from damaged cells, active release of ATP also occurs through exocytic granules, microvesicles and various transporters located on the tumor cells. Moreover, this nucleotide can be released by neighboring cells in the TME, such as mesenchymal stem cells (MSCs) or cancer-associated fibroblasts (CAFs). High concentrations of ATP are able to activate P2X7 receptors, which after prolonged activation are able to create a non-selective pore. However, the ability to create the pore depends on P2X7R variants, and additionally some established therapies can interfere with receptor expression or activation. We also postulate for a critical role of ecto-nucleotidases and ecto-kinases on MSC or CAF surface which, by orchestrating a fine-tune regulation of nucleotides concentrations, are integrally involved in modulation and diversification of purinergic signals. Since eATP in TME can modify cancer cell progression and interfere with administrated anti-cancer drugs, therefore, all aspects of ATP-mediated signaling need to be thoroughly evaluated before considering them as a part of anti-cancer therapy.

Summing up, all elements of purinergic system (nucleotides, P2 receptors, and nucleotide metabolizing enzymes) deserve continued scientific interest as the critical factors modulating cancer progression, and as targets for modern adjunctive therapies.

**New advance on the role of the ionotropic receptor P2X4 in the release of extracellular vesicles (EVs) release from mammary cancer cells and consequences on aggressiveness**

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Extracellular vesicles (EVs) are cell-derived particles including exosomes (50-150 nm), microvesicles (100-1000 nm) and apoptotic bodies (1–5 µm), which differ by their origins, sizes and compositions. Exosomes are a subtype of nanovesicles released by the fusion of Multivesicular Bodies (MVBs) with the cell membrane, well-known to be involved in intercellular communication and cancer progression. The molecular mechanism by which exosomes are released into the extracellular space still remains under controversary discussion. Therefore, it is highly recommended to investigate the key players that regulate the exosomal release and to develop or discover a molecular therapeutic candidate in the field of cancer In our previous study, we have generated a stable clone of mammary 4T1 cancer cell line that lack the expression of P2X4 (4T1-CR4) using CRISPR-Cas9 technology compared to a null-target 4T1-CTL cell line. EVs from conditioned medium of both cell lines were purified by differential ultracentrifugation, characterized by scanning electron microscopy (SEM), nanoparticle tracking analysis (NTA) and western blotting. Electron microsocpic images displayed an increased number of MVBs-like structures in 4T1-CR4 cells, which could a sign of intracellular accumulation of exosomes. NTA indicated that the release of CD9+ EVs was increased in 4T1-CR4 cells compared to control cells (4T1-CTL). These results were presented in a previous COST action (Pisa, 2023) showed a role of P2X4 in the endosomal pathway, mainly in the degradation of exosomes affecting their composition and consequences on invasive properties of recipient cells.

Our new experiment indicated that 4T1-CTL cell treated with the P2X4 antagonist 5-(3-Bromophenyl)-1,3-dihydro-2H-Benzofuro[3,2-e]-1,4-diazepin-2-one (5-BDBD) under hypoxia resulted in a significant increase of CD9+ EVs release. In addition, Inhibition of P2X4 expression drastically changed the expression profile of certain RAB proteins that are involved in MVBs trafficking. Proteomics data analysis from purified 4T1-CR4-EVs revealed an increase of several protein markers that are implicated in the early endosomal pathways (GO:2000643; GO:1902966). To investigate the role of P2X4 on the invasive property of 4T1 recipient cells, 4T1-CTL will be treated with EVs derived from 4T1 cells expressing or not P2X4. In conclusion, our results shed the light on the implication of P2X4 in the EVs release as well as in their molecular composition by impairing the exosomal degradation in breast cancer cells.

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**Purinergic Signalling in Breast Cancer: crosstalk between *de novo* and salvage nucleotide biosynthetic pathways.**

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Increased intracellular levels of nucleotides are required to sustain cell proliferation and tumour growth. Inosine 5'-monophosphate dehydrogenase (IMPDH) and Purine Nucleoside Phosphorylase (PNP) mediate GTP synthesis via *de novo* and purine salvage pathways, respectively. A number of IMPDH and PNP inhibitors underwent clinical trials for the treatment of several diseases, including cancer. In this study, triple-negative (MDA-MB-231) and ER-expressing (MCF7) human breast cancer cells were characterised for the expression of PNP and IMPDH vs. non-tumorigenic MCF12A mammary cells. Shotgun proteomics and label-free quantification by LC-MS/MS revealed different proteomic signatures of the mentioned cell lines under normoxic or hypoxic conditions. Noteworthy, cell treatment with IMPDH inhibitor (mycophenolic acid, MPA) or PNP inhibitor (forodesine), affected the pattern of proteins associated with cell growth/migration, immune response, oxidative stress, apoptosis and oncogenic signalling pathways. Both tumorigenic and normal cells treated with MPA 5 µM showed decreased proliferation, assessed by xCELLigence™ device, while forodesine 2 µM reduced MDA-MB-231 and MCF12A cell proliferation, while increased MCF7 viability. Supplementation with guanosine, precursor of GTP, partially recovered cell viability. Accordingly, cell pre-treatment with specific inhibitor of equilibrative nucleoside transporters (NBMPR 20 µM), reduced cell proliferation likely by inhibiting nucleoside uptake and the following nucleotide biosynthesis. Our findings suggest the existence of a fine-tuned nucleotide biosynthetic pathway which might be exploited as novel druggable target affecting tumour progression.

**Synthesis of novel bioactive molecules using visible light promoted photoredox chemistry in batch and microflow conditions**

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Even though significant progress has been achieved in the synthesis of biologically active molecules and potential pharmaceuticals, more efficient, selective and greener alternatives to many common synthetic processes still remain elusive. Catalysis is a key tool to render processes greener, more efficient and cheaper, paving the way towardsmore atom-economical transformations. Lately, the application of orgnocatalysis, photoredox and microfluidic chemistry have been a very promising strategies in this regard.

We have been optimizing cross dehydrogenative couplings of biologically privileged structures, tetrahydroisoquinolines using microfluidic chemistry and merger of photoredox and organocatalysis in the C1 functionalizations of tetrahydroisoquinolines,[1,2] valuable fine chemicals with important biological and pharmaceutical effects. Use of microreactors allowed shorter reaction times, superb yields, lower energy consumption, higher process efficiency and decreased waste generation compared to standard batch conditions. We have tested three types of custom made microreactors glass/silicon, FEP tube and PDMS microrectors that were optimized to perform synthesis of desired materials in the most efficient manner. Synthesized THIQ molecules showed potent anticholinergic and anti-cancerogenic properties and proved to be good DNAse I inhibitors.[3,4]

On the other hand, organofluorine compounds are also very important from the medicinal chemistry point of view. It is generally difficult to construct quaternary chiral carbon centers, and it is a synthetic challenge to develop a practical method for the stereoselective construction of a fluorinated quaternary chiral center with excellent stereoselectivity. We developed organocatalytic, asymmetric Diels–Alder reaction of $\alpha$-fluoro $\alpha,\beta$-unsaturated aldehydes using perchloric acid salt of diarylprolinol silyl ether as an organocatalyst, in water as a reaction medium. Excellent exo-selectivities and enantioselectivities were obtained with a generation of a fluorinated quaternary chiral center.[5]

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**Purinergic Signaling: from neurogenesis to neurodegeneration**

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Purinergic receptors have been shown to be important for development and tissue regeneration. Here, we have explored the participation of purinergic receptors in brain diseases, such as Parkinson´s and Huntington´s disease, using in vitro and in vivo models. Purinergic P2Y2 and P2X7 receptors have been studied in our laboratory regarding their roles in neural proliferation differentiation of stem cell models. P2Y2 and P2X7 receptors have antagonistic roles in this context with the P2Y2 receptor triggering neuronal fate determination and the P2X7 receptor promoting gliogenesis. We have also linked these receptors to neurodegeneration and provide novel promising targets for therapeutic approaches.

Parkinson's disease is a neurodegenerative disorder characterized by dopaminergic neuron death and decreased dopamine availability in the substantia nigra and striatum. Unilateral dopaminergic neuron degeneration was induced in the rat model by 6OH-dopamine (6OHDA) injection. Lesion establishment was confirmed after one week by rotational tests for confirming hemiparkinsonian behavior and by immunohistochemical analysis. P2X7 receptor blockade was investigated for reversing hemiparkinsonian behavior and dopaminergic neuron deficits in this animal model. P2X7 receptor antagonism with Brilliant Blue G reestablished dopaminergic ramifications in the striatum of rats injured with 6-OHDA following a period from 7 days on. Further, P2X7 receptor blockade prevented dopaminergic neuron death in the substantia nigra of 6-OHDA-injured rats. Moreover, both treatments were accompanied by a reduction of microglial activation in the substantia nigra. Altogether, antagonism of P2X7 receptors mediated neuroregenerative effects, respectively, possibly mediated through modulation of neuroinflammatory responses.

Huntington's disease (HD), an autosomal dominant inherited disease caused by at least 35 repetitions of the N-terminal CAG trinucleotide (glutamine) in the Huntington's gene, results in the loss of basal ganglia GABAergic neurons leading to motor, mood and cognition impairment as well as to uncontrolled movements. HD was modeled in vitro using CRISPR-Cas9 modified embryonic stem and HD patient-iPS cells induced to neuronal differentiation. We show that P2Y2 receptor activation promotes cell fate commitment to GABAergic neurons, while such neuronal fate determination did not occur in conditions of P2X7 receptor activation.

In summary, the here shown results of in vitro and in vivo studies point at the importance of purinergic receptors in neurodegeneration and open avenues for novel therapeutic applications involving the purinergic system.

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